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SERUM PROTEINS OF SWINE DURING HYPERIMMUNIZATION AGAINST SWINE PEST

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SERUM PROTEINS OF SWINE DURING HYPERIMMUNIZATION AGAINST SWINE PEST

/Following is the translation of an article by J. Poul in the French-language publication Archives de l'Institut Pasteur d'Algérie (Archives of the Algerian Pasteur Institute), Vol 40, No 1, Algiers, March 1962, pp 44-52./

During the operations required by the preparation of homologous anti-swine-pest serum intended for the vaccination of swine by the so-called "sero-inoculation" method, we were able to follow, in twelve serum-producing pigs, from the beginning to the end of hyperimmunization, the variations in the values of their serum proteins from both the qualitative and the quantitative point of view. The dosages of total proteins were prepared by the cold precipitation method with a mixture of equal parts of 95% alcohol and acetone /See NOTE/; electrophoresis of the serum on paper was accomplished by means of the Macheboeuf and Rebeyrotte apparatus. Since the serums studied were few in number (twelve), we believed it necessary to interpret the results obtained with statistical methods.

(/NOTE/: We are very grateful to Mme. Bats-Maillet, of the chemical laboratory of the Algerian Pasteur Institute, for having had the goodness to prepare these dosages.)

The sequence order of the operations may be summarized in the following manner, which shows the technique for preparing the anti-swine-pest serum used at the Algerian Pasteur Institute (2).

Day 0 -- Electrophoresis. Dosage of whole serum proteins.
Day 1 -- Sero-inoculation of the 12 pigs: 1/20 cc of virulent pest blood; 1 cc of serum per kg of live pig.
Day 16 -- 2nd electrophoresis. Dosage of total proteins.
Day 17 -- 1 cc of virulent pest blood, subcutaneously.
Day 35 -- 3rd electrophoresis. Dosage of total proteins; 10 cc of virulent blood subcutaneously.
Day 56 -- 10 cc of virulent pest blood.
Day 58 -- 4th electrophoresis. Dosage of total proteins.
Day 73 -- 300 cc of virulent pest blood peritoneally; 60 cc of ground pestiferous pig organs, subcutaneously.
Day 87 -- 5th electrophoresis. Total bleeding after puncturing the carotid. No dosage of total proteins was administered.

I. TECHNIQUES USED.

a. Dosage of total proteins. The method of dosage of the total serum proteins is a weight method, after cold precipitation with a mixture of equal parts of 95% alcohol and acetone (1). It provides slightly higher

Results Obtained

	Protéines totales g ‰	Albumine %	Albumine g ‰	α Globulines %	α Globulines g ‰	β Globulines %	β Globulines g ‰	γ Globulines %	γ Globulines g ‰
J 0	62,1 ± 3,5	19,8 ± 3,5	12,3 ± 2,2	26,9 ± 2,2	16,9 ± 1,4	21,7 ± 1,9	13,6 ± 1,2	30,6 ± 3	19,0 ± 1,9
J 16	74,0 ± 2,4	24,2 ± 3,7	17,9 ± 2,7	29,1 ±2,2	22 ± 1,6	19,7 ± 2,4	14,6 ± 1,8	26 ± 2,2	19,2 ± 1,6
J 35	74,2 ± 2,2	25,8 ± 4,4	19,1 ± 3,3	23,9 ± 2,8	18,2 ± 2,2	20,1 ± 2,2	14,9 ± 1,6	29,5 ± 3	21,9 ± 2,2
J 58	73,3 ± 2,2	25,6 ± 2,4	19,3 ± 2,8	22 ± 3	17,0 ± 2,2	25,0 ± 4	18,8 ± 3	26,5 ± 3	20,0 ± 2,2
J 87		31,5 ± 3		22,5 ± 2,8		20,5 ± 2,2		25 ± 2,4	

The figures which indicate the averages are followed by the limits of error of the average, computed for a 95 % probability. which means (L. Lison) (13) that we have 95 chances out of 100 of not being wrong in stating that the true average is included within the limits given.

Legend : J = day; Protéines totales = total proteins; Albumine= albumin; Globulines = globulins; 0/00 = per liter.
[NOTE: In the above table and wherever else occurring in the article, replace the comma in numerical values with a decimal point.]

than normal values, from 1% to 5%, as a result of the precipitation, in addition to true proteins, of similar substances that probably are polypeptides.

b. Electrophoresis. The swine serum proteins were studied by means of the Macheboeuf and Rebeyrotte (9) electrorheophoresis apparatus, with the following characteristics: tension on the paper contacts: 10 volts/cm, current intensity = 0.5 mA/cm, veronal buffer with pH = 8.6 and an ionizing force = 0.05; duration of the electrophoresis: 5 hours. Development was accomplished with bromophenol blue (slow method); the comparison of the strips colored like that was facilitated by recording them, in diagrammatic form, with the Leres automatic recording photometer. There are numerous causes of error (11); nevertheless, by using a large tank and a paper (Arches 304) that allows the simultaneous electrophoresis of 12 serums we were able to work under the best conditions for comparing the different diagrams.

c. Interpretation of the results. Since the number of serums studied was small (twelve), we believed it necessary, in order to avoid errors due to individual responses, to compute, in addition to the typical deviation, the limits of error of each average for a 95% probability, a practice currently admitted for this type of experiments.

II. SERUM PROTEINS OF THE UNEXPOSED PIG

We assumed that on Day 0 the twelve pigs were unexposed and we considered that the average results obtained (followed by the typical deviation) were valid for the normal pig. We therefore have:

Total proteins: 62.1 g per liter ($\pm$5.5),
Albumin serum: 19.8 % ($\pm$5.5) or 12.5 per liter ($\pm$3.5),
Globulin serum: α : 26.9 % ($\pm$3.6) or 16.9 g per liter ($\pm$2.2),
β : 21.7 % ($\pm$2.9) or 13.6 g per liter ($\pm$1.9),
γ : 30.6 % ($\pm$4.9) or 19.0 g per liter ($\pm$2.3).

Let us recall that for a reliability coefficient of 95% we have a probability of 5 chances out of 100 of finding an individual that deviates from the average by more than twice the typical deviation. We can state, then, that in normal swine serum the proteins have the following values:

Total proteins: 62.1 $\pm$ (1.96 X 5.5), or included between 51.4 and 72.8 g per liter.
Albumin serum: 19.8 % $\pm$ (1.96 X 5.5), or included between 9.1 and 30.5 % or 12.5 g per liter $\pm$ (1.96 X 3.5), or included between 5.7 and 19.3 g per liter.

Globulin serums:

α :	26.9 % ± (1.96 X 3.6)	or included between		20.0 and 33.8 %
	16.9 g per liter ± (1.96 X 2.2)	"	"	12.6 and 21.2 g per liter
β :	21.7 % ± (1.96 X 2.9)	"	"	16.1 and 27.3 %
	13.6 g per liter ± (1.96 X 1.9)	"	"	9.9 and 17.3 g per liter
γ :	30.6 % ± (1.96 X 4.9)	"	"	22 and 39.2 %
	19.0 g per liter ± (1.96 X 2.3)	"	"	14.5 and 23.5 g per liter

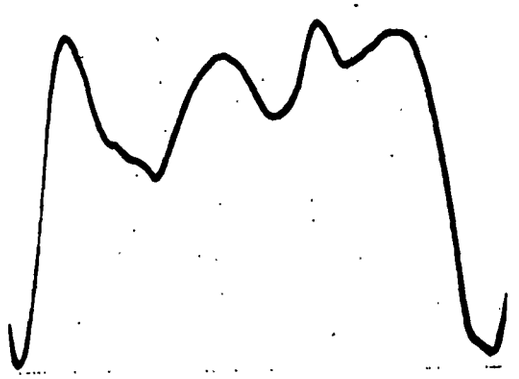

Diagram 1

As the above diagram (1) shows, normal swine serum proteins are characterized by four peaks perceptibly equal in size; the first peak represents the albumin fraction that migrates farther, then come, in decreasing order of migration velocity, α, β, and γ globulins. In every case the α globulins that are found close to the albumins are of little consequence and represent scarcely 1% of the total serum proteins.

Edsall (3) grants that the sizable variations in the protein composition of the serum of certain animal species are characteristic of them. Therefore he finds that the percentage of serum albumin in the pig oscillates between 46.8% and 64.4%.

J. A. Berger (6) indicates, still for the normal pig, the following values:

Albumins: 39%
Globulins: α : 19%; β : 21%; γ : 21%

For W. Boguth (7) electrophoresis of normal swine serum is characterized by:

Albumins: 44.8%
Globulins: α : 16.7%; β : 18.7%; γ : 19.8%

After studying 20 swine serums, H. Hill and G. Schumann (8) obtained the following results:

Albumins: 47.4% (41.8 to 55.4)
Globulins: α : 19.8% (16.7 to 21.7)
β : 14.7% (12.9 to 18.2)
γ : 18.4% (14.2 to 22.0)

whereas R. Panisset (10), averaging the four serums, indicates:

Albumins: 30.5%
Globulins: α : 22.8%
β : 19.3%
γ : 27.4%

P. Vignand (12) identifies the albumin fraction only by its greater migration velocity and, using the interpretation method indicated by Drevon, divides the globulin fraction of the serum into five equal parts, numbered from I to V, and thus he finds for normal swine serum:

Albumins: 13.5%
Globulins: I: 25.35%
II: 22.15%
III: 28.00%
IV: 17.40%
V: 7.05%

As for L. M. Knill, T. R. Podleski and W. A. Childs (14), the electrophoretic examination of the serum of healthy pigs allows them to divide up the various fractions found as follows:

Albumins: 46%
Globulins: α : 20%
β : 14.5%
γ : 19.5%

for a total proteins value equal to 7.4 g per 100 cc of serum.

III. VARIATIONS IN SWINE SERUM PROTEINS DURING HYPERIMMUNIZATION AGAINST SWINE PEST.

1. Total proteins. Beginning with Day 16 and probably until Day 87 (although the dosage was not administered it, can reasonably be thought that it is so), the total proteins increased appreciably, because their value goes from 62 to 74 g per liter of serum, or an increase of 19.3%, and it holds there. It did not seem possible to us to explain this increase or even to consider a plausible hypothesis for it. We limit ourselves, therefore, to stating the fact.

2. Variations of the various fractions. We were expecting to find a modification of the globulins, especially γ or β globulins.

Now, an examination of the accompanying table lets us state that the values of the α, β and γ globulins did not vary in the twelve swine serums studied and in the same twelve serums of hyperimmunized pigs. On the contrary, there was a considerable increase, since it exceeds 50% for the albumins.

It cannot be believed that the anti-swine-pest antibody, prepared on the pig under the conditions that we indicated, might be a serum albumin, because G. Gayot (4) has already demonstrated that it was a globulin. Two explanations, then, come to mind:

either electrophoresis on paper is not a sufficiently sensitive technique to show up the eventual modifications of the globulins in the case studied;

or this modification affects the quality of the globulins rather than their quantity.

Therefore we had to verify whether the γ globulins (or 33 globulins) of the anti-swine-pest serum were really the supports of the neutralizing antibody; now, vaccination of the pig against swine pest by the sero-inoculation method consists of the inoculation of a fixed dose of diluted swine pest virus and of anti-serum on the basis of 1 cc per kg of live weight. Consequently, two series of sero-inoculations are administered, one of them with serum drawn on Days 0, 16, 35, 58 and 87, and the other with the γ globulins of the same serums.

These γ globulins were separated by precipitation with ammonium sulphate at a saturation of 33%. A dialysis with distilled water allowed the insoluble fraction of the euglobulin that was eliminated by centrifugation to precipitate. We sero-inoculated pigs in this manner after having brought the solution of 33 globulin back to its initial volume, so that the pigs received the same volume of serum or of γ globulin. A small amount of each solution was drawn off to check its homogeneity by electrophoresis on paper.

Eleven pigs were used: one, as a control, received only virus. The rest received, in addition to the same dose of virus as the control, 1 cc per kg, either of serum or of γ globulin drawn on different days.

Twenty-six days after their sero-inoculation, the surviving pigs received subcutaneously a test inoculation composed of 1 cc of very virulent pest blood, Casa strain. All of them not only resisted, but did not present either hyperthermia or any clinical signs in the 10 days that followed.

The complete results may be summarized as follows:

Control pig: died of swine pest 8 days after inoculation.

Day 0: serum pig, died of pest in 9 days.
γ pig, died of pest in 11 days.

Day 16: serum pig, died of pest in 17 days.
γ pig, died of pest in 13 days.

Day 35: serum pig, both resisted and are immunized
γ pig, against the pest.

Day 56: serum pig, resisted and is immunized against the pest.
γ pig, died of swine pest in 20 days /See NOTE/.

Day 87: serum pig, both resisted and are immunized
γ pig, against the pest.

(/NOTE/: The death of this pig, due certainly to a more pronounced individual sensitivity, takes nothing away from the value of the experiment.)

Therefore, the neutralizing antibody is really a γ globulin that electrophoresis on paper, in comparison with normal pig serum, did not show up.

It even seems that it may be a pseudoglobulin. In fact, we noticed, during the dialysis with distilled water that allows the separation of pseudoglobulins, after precipitation with ammonium sulphate at a saturation of 33%, that the precipitate of euglobulins was greater with the normal pig serum than with hyperimmunized pig serum. Everything seemed to take place as if, with the total amount of 33 globulins remaining unchanged, the insoluble euglobulin in the distilled water, present in the normal pig serum, had been transformed into a pseudoglobulin antibody, present in the hyperimmunized pig serum.

CONCLUSION

1. The study of serum proteins in 12 pigs gave us, thanks to electrophoresis on paper, the following results (the values between parentheses are the typical deviation):

Total proteins:	62.1 g per liter	(±5.5)
Albumins :	12.5 g per liter	(±3.5)
Globulins :	16.9 g per liter	(±2.2)
	13.6 g per liter	(±1.9)
	19.0 g per liter	(±2.3)

The albumin/globulin ratio, calculated in accordance with the electrophoresis diagram on paper, is very low, equal to 0.25.

2. In the serum of the pigs hyperimmunized against swine pest for the purpose of preparing an anti-swine-pest serum, electrophoresis on paper allowed us to observe, in the serum of the same 12 pigs, a 50% increase in the albumin fraction and an almost 20% increase in serum proteins.

3. The neutralizing antibody is a pseudoglobulin.

4. Electrophoresis on paper cannot cause this pseudoglobulin to show up. Moreover, R. Raynaud, J. R. d'Eshouges, R. Vargues and P. Pasquet (5) have already shown, by comparing the results obtained by a Tiselius electrophoresis, electrophoresis on paper and reticuloendothial index, that "pseudoglobulins showed up only exceptionally to disturb the electrophoresis on paper tracings". Therefore it is not surprising that the anti-swine-pest antibody was not revealed by this process.

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